IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Robert C. Ladner *et al.* Art Unit : 1639
Serial No. : 10/656,350 Examiner : Jeffrey S. Lundgren
Filed : September 5, 2003
Title : DISPLAY LIBRARY PROCESS

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 CFR 1.131

I, Kristin Rookey, a citizen of the United States, residing at 14 Hancock Street, Revere, MA 02151, U.S.A., hereby declare as follows:

1. I am an inventor of the subject matter disclosed and claimed in the above-referenced United States Patent Application.

2. I am familiar with the present claims of the application, which are directed to, *inter alia*, methods of selecting phage that encode a target binding protein from a plurality of display phage.

3. Prior to June 21, 2002, I had reduced my invention to practice as described and claimed in the above-identified application in this country, a NAFTA country or WTO country, as evidenced below.

4. I submit herewith Exhibits B-D evidence showing reduction to practice of the claimed invention prior to the June 21, 2002. The date on each page is redacted. Each page is dated prior to June 21, 2002.

   Exhibit B is an excerpt from my laboratory notebook showing experiments that included three rounds of phage selection.

   Exhibit C is an entry from my laboratory notebook with experiments showing multiple rounds of phage selection, followed by tests of specificity of selected positives by ELISA.

   Exhibit D is an entry from my laboratory notebooks showing the diversity of selected positive clones (as seen on the agarose gels).

5. In sum, I submit herewith evidence (Exhibits B-D) that shows reduction to practice of the claimed methods prior to June 21, 2002.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, under Title 18 § 1001 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

5-4-07
Date

Kristin Rookey

21612335.doc

1613-036 DATE SUBJECT Fast Selection - SA

CONTINUED FROM

Object Test out a new method of selections whereby all 3 rounds of selections are done in 1 day

1. block 100 ml Dynal M280 SA magnetic beads w/ 2% MPBS - total Vol = 500 ml (3 separate rxns of 100 ml each)
2. α1' phage
   K = 1.5×10^{10}/ml
   L = 1.5×10^{10}/ml
   10 ml of each (3×10^{10} total) to blocked SA beads. Raise Vol to 500 ml w/ 2% MPBS
   60 min RT
3. WASH 3X w/ PBS + .01% Tween
4. Add 500 ml of XL1 Blue Murf' cells $OD_{600} = .455$
   transfer to 50 ml conical
   #1 37°C 20 min
   30°C 25 min
   #2 37°C 45 min
   #3 30°C 45 min
   AT 15 min add 5 ml of 100 mM IPTG to get 1 mM IPTG
5. Remove bacteria, Titer on XL41 & XL0 10 ml & .1 .01 ml
6. WASH 3X PBS + .01% Tween
7. Repeat steps 4-6 2 more times
8. expand the 500 ml XL1 Blue Murf' output cultures to 10 ml 2XYT + 1 mM IPTG 30°C O/N

Count Colonies & ppfu

37°C/30°C Round #1 1168 on .01

5.84×10^7 ppfu

Input - 3×10^{11} fOI = 1.95×10^{-4}

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CONTINUED FROM

37°C/30°C Round 2 101 on .1 ml

5.1×10^5 cfu

input = 5.84×10^7 cfu $FOI = 8.8 \times 10^{-3}$

rens each)

Round 3 146 on 1 ml

7.3×10^4 cfu

$FOI = 1.4 \times 10^{-1}$

37°C 45 min

Round 1 1602 on .01 ml

8×10^7 cfu

$FOI = 2.7 \times 10^{-4}$

Round 2 241 on .1 ml

1.2×10^6 cfu

$FOI = 6.5 \times 10^{-2}$

Round 3 107 on 1 ml

5.4×10^4 cfu

$FOI = 4.5 \times 10^{-2}$

1 ml DOT

.1 .01 ml

30°C 45 min

es

Round 1 1026 on .01 ml

5.13×10^7 cfu

$FOI = 1.7 \times 10^{-4}$

Round 2 1500 on 1 ml

7.6×10^5 cfu

$FOI = 1.2 \times 10^{-2}$

Round 3 95 on 1 ml

4.75×10^4 cfu

$FOI = 6.3 \times 10^{-2}$

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pfu

37°/30°C Round 1 5×10^8 pfu
2 2×10^7 pfu foI = 4×10^{-2}
3 4.3×10^6 pfu foI = 2.1×10^{-1}

39°C Round 1 5×10^8 pfu
2 2.6×10^7 pfu foI = 5.2×10^{-2}
3 2.1×10^6 pfu foI = 7.8×10^{-2}

30°C Round 1 5×10^8 pfu
2 2.5×10^7 pfu foI = 5×10^{-2}
3 3.35×10^6 pfu foI = 1.3×10^{-1}

note - enrichment is positive between rounds
- attempt additional targets ~~for participant on the patient~~ for
- pick plaques & attempt PCRs

24 hpt + 1 mM IPTG 30°C in 96 well plate

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DATE SUBJECT Fast Screen Fitc 1613-039

CONTINUED FROM

Object: 2nd attempt at Fast Screen w/ Fitc on SA beads

1. block 100 ul of Dynal M280's SA magnetic beads
   2% MPBS 60 min
   (rinse w/ MPBS 2x prior to blocking)
2. 10 ul of K (1.5 x 10^{10}/ul)
   10 ul of L (1.5 x 10^{10}/ul)

   1.43 ul Fitc BSA .7 ug/ul

   Raise vol to 50 ul in 4% MPBS

   60 min on rotator
3. Remove block from beads
4. add 50 ul of Fitc-BSA/phage to beads
   raise vol to 500 ul w/ 2% MPBS
   30 min on rotator
5. Wash beads 5x PBS + .01% tween
6. add 500 ul XL1Blue MRF' OD_{600} = .5
   - move to 50 ml conical
   37°C 20 min
   @ 15 min add 5 ul 100 mM DTB
   30°C 25 min
7. Remove bacteria
   titer 100 ul 10^{-1} -2 -3 on DL41
   expand bacteria to 10 ml culture 2xYT + 1 mM DTB
   30°C O/N
8. Wash beads 3x PBS + .01% tween

Repeat 6-7 2 more times

Count cfu	total cfu	EOF
R1 10^{-3} = 800 cfu	4 x 10^6	1.3 x 10^{-5}
R2 10^{-3} = 156	7.8 x 10^5	2 x 10^{-1}
R3 10^{-3} = 25	1.25 x 10^5	1.6 x 10^{-1}

- expand colonies for ELISAs
  30°C O/N in 2xYT + 1 mM DTB 96 well plate

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CONTINUED FROM

Object: test Fast Screen phage output for Specificity

1. Coat 6X Immulon 2 100ng GAR BSA/100ml 100mM Sod. Bicarb pH 8.5
   6X " 100ng SA/100ml "
   O/N 4°C
2. block 1 hour 2% MBS 100ml/well
3. WASH 3X PBS/.05% Tween
4. Add 100ml Supernatant each well on SA & on GAR
   60min RT
5. WASH
6. 100ml/well anti-M13-HRP Amersham 1/5000 60min
7. WASH
8. TMB

pick 12 FAR positives from K3 to continue with
B9 D8 D10 G3 G9 F9 F10 G1 G10 H1 H4 H10

pick 9 SA positives from K2
A7 B8 C3 C10 G5 G11 F4 F8 F10

See page 42

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1613-042 DATE SUBJECT

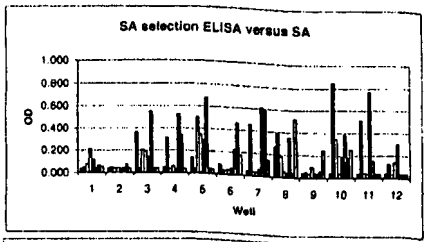
CONTINUED FROM

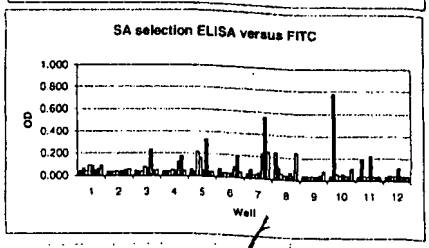
SA on SA

0.035	0.039	0.368	0.051	0.140	0.068	0.038	0.218	0.043	0.043	0.042	0.043
0.050	0.053	0.039	0.310	0.038	0.037	0.457	0.384	0.051	0.848	0.511	0.127
0.078	0.039	0.208	0.035	0.504	0.056	0.036	0.187	0.038	0.343	0.048	0.037
0.214	0.047	0.184	0.060	0.345	0.039	0.037	0.053	0.096	0.040	0.039	0.142
0.126	0.037	0.142	0.036	0.295	0.052	0.059	0.044	0.046	0.185	0.769	0.302
0.042	0.040	0.550	0.526	0.682	0.220	0.613	0.353	0.030	0.380	0.154	0.038
0.070	0.077	0.048	0.340	0.040	0.464	0.597	0.044	0.058	0.180	0.038	0.036
0.056	0.042	0.041	0.037	0.040	0.180	0.145	0.522	0.245	0.252	0.038	0.040

SA on FITC

0.040	0.037	0.044	0.038	0.058	0.071	0.039	0.245	0.040	0.038	0.039	0.039
0.065	0.037	0.037	0.044	0.039	0.037	0.078	0.105	0.040	0.776	0.195	0.051
0.042	0.036	0.039	0.036	0.217	0.038	0.037	0.057	0.039	0.061	0.044	0.041
0.094	0.040	0.090	0.057	0.188	0.038	0.038	0.041	0.042	0.043	0.040	0.048
0.089	0.036	0.067	0.043	0.047	0.040	0.053	0.039	0.038	0.059	0.222	0.117
0.044	0.041	0.233	0.127	0.332	0.096	0.228	0.064	0.039	0.044	0.042	0.045
0.060	0.053	0.047	0.178	0.044	0.190	0.557	0.042	0.047	0.040	0.037	0.040
0.092	0.058	0.049	0.043	0.043	0.057	0.245	0.246	0.085	0.109	0.039	0.038



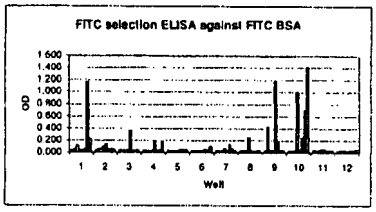


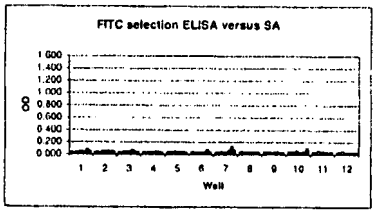
FitC on FitC

0.041	0.041	0.043	0.042	0.042	0.044	0.043	0.039	0.041	0.040	0.054	0.039
0.066	0.065	0.040	0.041	0.044	0.038	0.039	0.039	0.434	0.037	0.035	0.037
0.116	0.064	0.045	0.037	0.039	0.039	0.039	0.041	0.044	0.039	0.045	0.042
0.042	0.099	0.045	0.040	0.044	0.039	0.074	0.249	0.040	1.010	0.051	0.034
0.041	0.138	0.363	0.194	0.042	0.042	0.043	0.039	1.179	0.051	0.066	0.034
0.064	0.055	0.039	0.042	0.058	0.059	0.137	0.042	0.192	0.249	0.040	0.037
1.165	0.060	0.039	0.053	0.050	0.053	0.072	0.039	0.048	0.714	0.042	0.035
0.223	0.054	0.042	0.188	0.050	0.108	0.038	0.040	0.043	1.410	0.044	0.059

FitC on SA

0.040	0.038	0.039	0.038	0.043	0.043	0.041	0.048	0.051	0.055	0.044	0.041
0.037	0.042	0.037	0.036	0.039	0.042	0.041	0.047	0.049	0.046	0.043	0.046
0.040	0.040	0.050	0.044	0.039	0.037	0.049	0.038	0.043	0.042	0.058	0.055
0.050	0.081	0.048	0.038	0.053	0.045	0.042	0.041	0.037	0.068	0.048	0.036
0.056	0.064	0.056	0.048	0.048	0.042	0.038	0.042	0.044	0.041	0.049	0.040
0.044	0.066	0.076	0.056	0.049	0.043	0.059	0.042	0.054	0.041	0.044	0.037
0.061	0.055	0.051	0.045	0.044	0.061	0.138	0.048	0.039	0.048	0.036	0.036
0.058	0.052	0.043	0.042	0.039	0.045	0.078	0.044	0.038	0.101	0.040	0.040





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SUBJECT Fingerprint TR/SA binders

CONTINUED FROM

Object - fingerprint 12 TR positives & 9 SA positives w/ BSTNI

1. Colony PCR

10x PCR buffer II	10	95°C 5m
10mM dNTPs	8	95°C 30s
25mM $MgCl_2$	8	60°C 30s
ONB fab HindIII 100 pmol/µl	.1	72°C 1m 30s
ONT fab AflIII 100 pmol/µl	.1	72°C 5m
TAQ	.5	
dH_2O	71.5	
	100µl	

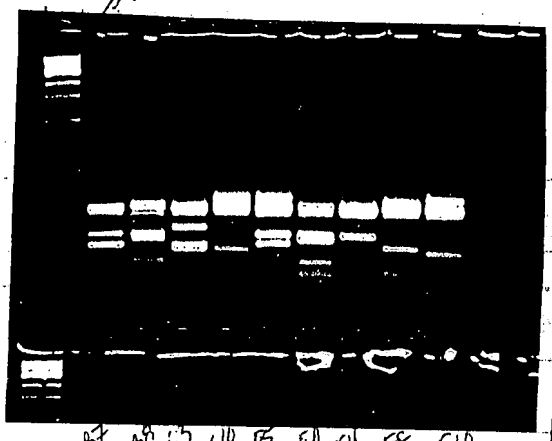
28x

2. Digest w/ BSTNI

10x BSA	2 µl
10x NEB2	2.5 µl
BSTNI 10U/µl	1 µl
PCR prod	20 µl
	25 µl 60°C 1hour

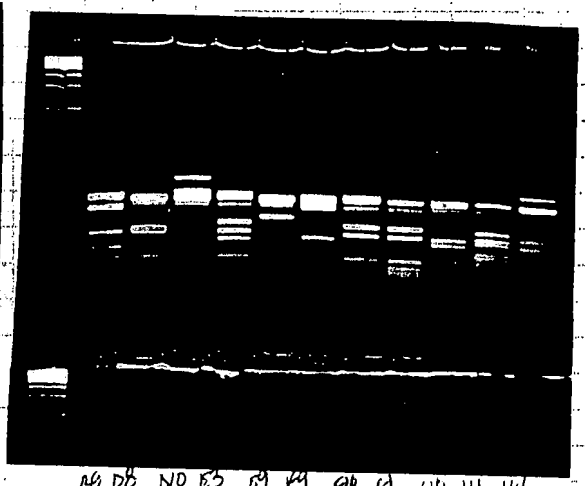
3. 3% Agarose gel -load all

SA:



B7 B8 C3 C10 E5 E11 F4 F8 F10

TR:



A9 B8 D10 E5 G1 H4 A10 G1 G10 H1 H4

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